

Methods of Plant Culture and Plant Analysis

Plant-Culture Techniques Adapted to Salt-Tolerance Investigations

There are certain aspects of salt-tolerance investigations involving extensive plant breeding and selection programs that are being conducted by crop specialists at a number of agricultural experiment stations. Furthermore, since salt tolerance of plants may be influenced profoundly by climatic conditions, investigators in a given region may find it desirable to undertake salt-tolerance studies under local climatic conditions in order to determine the crops best suited to their region. In anticipation of a continued interest in such breeding and testing programs, techniques used in such studies at the Salinity Laboratory are included in this handbook.

(50) Artificially Salinized Field Plots

Information is needed regarding the response of crop plants to adverse saline conditions as related to climatic factors, planting techniques, and cultural practices used in the field. However, field observations relating crop growth to the salinity of the soil are difficult to evaluate, because of the wide range of salt concentrations frequently encountered within a small area in the field. In order to obtain information on these aspects of salt tolerance, an artificially salinized field-plot technique may be employed (Wadleigh and Fireman, 1949). Small, 14-ft. square plots are used, and each crop is managed according to practices generally followed in the principal regions where it is grown under irrigation. Density of stand, spacing, fertilizer practice, irrigation methods, and other conditions are all reproduced as closely as possible. The salt tolerance of field, vegetables, forage, and tree crops may be studied by this technique.

In the preparation of plots, careful leveling is necessary to insure uniform distribution and penetration of the salinizing water, and all parts of a basin-irrigated plot should be brought to within $\frac{1}{4}$ in. of the mean plot level. Similar precautions should be used where furrow irrigation is to be practiced so that all bed surfaces and furrows will be at uniform levels. The salinizing waters can be restricted to the plots by borders; and, if the plots are closely spaced, the borders can be supplemented with 6-in. boards around the plot. Asphalt roofing paper 18 in. wide can be attached to

the boards and buried to a 12-in. depth. If the soil is sufficiently permeable and adequate irrigations are applied, salt concentration in the plot tends to reach a steady value following the first several irrigations with a minimum of variability within the plot.

In order to avoid the development of alkali conditions in the soil, it has been standard practice to add salts to the irrigation water as equal parts of sodium chloride and calcium chloride. This is readily accomplished for small plots by dissolving the desired amount of salts in water in a galvanized tank and mixing with the aid of a circulating pump. Salinity levels commonly employed in salt-tolerance tests are 0, 3,000, 6,000, and 9,000 p. p. m. of added salts, although a more dilute series may be desirable for the more salt-sensitive crops, such as beans and fruit trees; and a more concentrated series for salt-tolerant crops, such as barley and beets. Salinization of the plots is begun after the seedlings or transplants are established because of the greater sensitivity of most crops to salinity during germination and immediately following transplanting. To avoid the shock, which too abrupt a change in soil salinity may induce in the plant, it is advisable to increase salinity stepwise during the first 3 or 4 salinizing irrigations. By applying relatively light irrigations at frequent intervals, the salinization of a series of plots may be completed in 7 to 10 days. However, owing to dilution of the salinizing water by residual soil moisture, a few additional salinizing irrigations may be required to establish a relatively constant concentration of salt in the plot.

Subsequent irrigations are usually applied at conventional frequencies, as judged by the condition of the control plot. The salt status of the salinized plots is determined periodically during the growth of the crop by taking soil samples at various depths in the beds and furrows and determining the conductivity of the saturation extract (EC_e). Yield data and observations on crop quality and composition can then be correlated with the salt status of the several plots in the series.

Plots treated in the above manner may be reused after leaching the salt from the soil. Although the plots remain nonalkali, recovery is facilitated by the addition of gypsum at the rate of 1 ton per acre. To insure continued uniformity of the plots with regard to soil structure (as measured by water penetration, aeration, and other properties), it is desirable to grow some salt-

tolerant grass following leaching and prior to reusing the plots.

The above procedure may be modified for certain purposes. For crops that may be specifically sensitive to high concentrations of soluble calcium or chloride, the composition of the added salt may be changed to vary the sodium: calcium ratio, or sulfate may be substituted for some or all of the chloride. The solubility of calcium sulfate is a limiting factor in making such substitutions, and the effect of high ratios of sodium to calcium on the exchangeable-sodium-percentage must be taken into account.

Inasmuch as this procedure does not include observations on the salt tolerance of the crop during germination, this must be determined separately, either by a laboratory technique (Ayers and Hayward, 1949) or by a modification of the artificially salinized field-plot technique. The plots may be salinized prior to planting with a series of waters of graded concentrations. Salinity levels are then determined by *EC_e* measurements, and the seeds are planted according to standard or experimental practice. Irrigating with water, usually much less saline than that used in salinizing the plots, will result in a redistribution of salt, especially where furrow irrigation is practiced. Germination counts will serve to delimit the critical level of salinity which can be tolerated by the crop at the time of planting under the specific cultural conditions employed.

(51) Drum Cultures

Drum cultures have been used extensively for studies of salt tolerance relating to the specific effects of various added salts and to the frequency of irrigation as it interacts with salinity in affecting the growth of plants (Wadleigh and coworkers, 1951; Wadleigh and Ayers, 1945). Since the number of treatments may be greatly increased with a minimum of space and effort by using drum cultures, this technique has a very definite place in salinity investigations.

Salinization of drum cultures requires a different technique from that employed with artificially salinized plots. Any salt added to the drums remains in the soil and is not moved downward past the root zone by subsequent irrigations as in field plots. It is, therefore, necessary to add the salt only in the initial irrigation of the drums or by mixing salt with the dry soil in amounts calculated to give the desired salinity levels. Thereafter, nonsaline water must be used in irrigating to avoid further increases in the salt concentration of the soil. However, repeated irrigations with nonsaline water will tend to leach the salt downward in the drum, and a very steep gradient in salt concentration will ensue.

In order to maintain a relatively uniform distribution of salt in the soil, it is necessary to irrigate alternately on the surface and by subirrigation. For subirrigation, provision is made for introducing the irrigation water into a layer of fine gravel in the bottom

of the drum before filling with soil. With alternate surface and subirrigation the distribution of the salt in the soil can be maintained more nearly uniform. Frequency of irrigation may be determined by daily weighings of the drum cultures and calculation of mean soil-moisture content. It is important to avoid overirrigation of the drums, since drainage, if permitted, will result in loss of salt; or, if drainage is prevented, the saturated soil condition will inhibit proper root activity.

(52) Sand and Water Cultures

Frequently, problems difficult to solve by soil-culture methods can be studied more satisfactorily by sand or water cultures, because the latter allow for a more precise control of the substrate. Salinity studies using sand or water cultures involve the addition of various salts to a base nutrient solution. The salts may be added in isosmotic concentration to facilitate comparisons of growth or in isoequivalent concentration to permit a readier comparison of accumulation of the elements in question. Occasionally, the two methods of adjusting salt concentration are combined to permit both isosmotic and isoequivalent comparisons, or a series of concentrations of each salt may be used, and the effect of osmotic pressure and equivalent concentration determined by graphical analysis of the data. By these techniques the effects of various ions, such as sodium, potassium, calcium, magnesium, chloride, sulfate, and bicarbonate, on the growth and composition of plants may be studied. In sand or water cultures the treatments are not altered as a result of cation exchange, nor is fluctuating moisture content a disturbing factor as it is in soil cultures.

For sand and water cultures, provision must be made for adequate nutrition by use of a base nutrient solution, proper control of the pH of the nutrient solution, and adequate aeration.

Methods of Plant Analysis

[See the introductory notes at the beginning of chapter 6]

(53) Sampling and Preparation of Plant Samples

In collecting plant samples for chemical analysis in connection with salinity studies, the usual care should be practiced to obtain material representative of the plant population. Since plant organs may differ markedly in their selectivity in accumulating various ions, it is frequently desirable to collect separate fractions of the various plant parts: leaves, stems, and roots. In taking leaf samples it is the usual practice to select mature, fully expanded leaves, avoiding senescent ones, since salt accumulation may vary with age of leaf. If some leaves are affected by leaf burn or other visual symptoms of salt injury, separate samples of affected and normal leaves may furnish data of diagnostic importance.

Remove surface contamination of the plant material by brushing and brief rinsing in distilled or demineralized water. If iron or other heavy metals are to be determined, rub the entire surface gently in 0.3 N hydrochloric acid and rinse. Dry the sample rapidly in a forced-draft oven at 70° C. Grind the sample in a Wiley mill, or by means of a mortar and pestle if metallic contamination is to be avoided. Mix well and store in tightly stoppered containers. Label each sample, giving information on species, plant part sampled, fresh weight, date, collector, and other pertinent data.

If soluble sap constituents are to be determined, freeze weighed amounts of fresh plant material in wide-mouthed jars and store at -10° C. To obtain sap, thaw the plant material quickly by immersing the container in running water, press at 10,000 to 15,000 lb. per sq. in. until the major portion of the sap has been released. Centrifuge the liquid 15 min. at $RCF=1,500$, pour off the supernatant liquid, and save for analysis. The expressed sap may be kept for several days in a refrigerator if a few drops of toluene are added, but analysis of the fresh sap is preferred. When the determination of insoluble constituents is desired, combine the residue from the centrifuge tube with the press cake, determine moisture content, and analyze for desired constituents. Correct these results on the basis of moisture content of the press cake and the concentration of soluble constituents of the sap.

Procedures for chemical analyses of plant materials routinely employed in salt-tolerance studies are described under Methods 54 to 62. Additional determinations of various plant constituents are sometimes desirable, depending upon the specific problems under investigation. Methods employed in studies of bicarbonate-induced chlorosis have been described by Wadleigh and Brown (1952).

(54) Ashing

(54a) Wet Digestion

This procedure is preferable to dry ignition, because of the possibility of loss of mineral constituents at high temperatures during dry ignition.

Reagents

- A. Nitric acid, conc.
- B. Perchloric acid, 72 percent.

Procedure

Transfer 1.000 gm. of dried plant material or an equivalent volume of sap (usually 10 ml.) to a 50-ml. beaker and add 20 ml. of reagent A. Cover with a watchglass and allow to stand until initial reactions subside. Heat until solid particles have nearly disappeared, cool, add 10 ml. of B. *Caution: Perchloric acid is explosive in presence of easily oxidizable organic matter.* Heat gently at first, then heat more vigorously until a clear, colorless solution results. Do not take to

dryness; discontinue heating when the volume is reduced to approximately 3 ml. Cool and transfer quantitatively to a 100-ml. volumetric flask, make to volume, mix, allow to stand overnight and filter through a dry filter paper without washing. Retain this solution and use for analyses as described under Methods 55 to 58 and 61.

Reference

Toth and associates (1948).

(54b) Magnesium Nitrate Ignition

Since the wet-digestion procedure does not quantitatively retain sulfur, magnesium nitrate ignition should be used in the determination of sulfur in plants. Total phosphorus may be determined in the ignited material as well, since phosphorus is quantitatively oxidized to phosphate by this ignition.

Reagents

A. Magnesium nitrate solution. Add 113 gm. of magnesium oxide to 300 ml. of water and stir to a paste. Add nitric acid (1+1) until the magnesium oxide is in solution. Add a little excess magnesium oxide and boil; filter and dilute to 1 liter.

B. Hydrochloric acid (1+1).

Procedure

Weigh 2.000 gm. of the sample and transfer into a large porcelain crucible or casserole. Add 10 ml. of reagent A, taking care that all the material is brought in contact with the solution, and heat gently on a hot plate to 180° C. until the reaction is complete. Transfer the crucible while hot to an electric muffle and allow to remain at low heat (muffle must not show any red) until the charge is thoroughly oxidized. No black particles should remain. (It may be necessary to break up the charge and return to the muffle.) Remove from the muffle and allow to cool. Moisten the ash with water and add 10 ml. of B, which should provide an excess of acid. Evaporate to dryness, moisten with B, and again evaporate to dryness to dehydrate silica. Add 5 ml. of B and sufficient water to bring the salts into solution. Allow to stand on the steam bath until solution is complete. Filter, wash, and make to 100 ml. with water. Retain this solution for analysis of sulfur and phosphorus according to Methods 60 and 61.

Reference

Association of Official Agricultural Chemists (1950, 2.8 (e), p. 8, and 6.35, p. 104)

(55) Calcium

(55a) Calcium by Flame Photometer

Apparatus

Perkin-Elmer model 52 flame photometer with acetylene or propane burner.

Reagents

A. Lithium chloride, 0.05 N. Dissolve 2.12 gm. of lithium chloride in water and make to 1 liter.

B. Sodium chloride, 5.00 meq./l. Dissolve 0.2922 gm. of dry sodium chloride in water and make to 1 liter.

C. Potassium chloride, 12.5 meq./l. Dissolve 0.9320 gm. of dry potassium chloride in water and make to 1 liter.

D. Calcium chloride, 50.0 meq./l. Dissolve 2.503 gm. of pure calcium carbonate (calcite crystals) in 50 ml. of 3 N hydrochloric acid and dilute to 1 liter.

E. Magnesium chloride, 50.0 meq./l. Dissolve 5.1 gm. of $MgCl_2 \cdot 6H_2O$ in approximately 900 ml. of water. Standardize by Method 78 and add the calculated amount of water to bring the magnesium concentration to 50.0 meq./l.

Remarks

The optimum concentration of lithium chloride for use as an internal standard varies with individual flame photometers but is usually 5 to 10 meq./l.

Interference by sodium, potassium, and magnesium is not an appreciable factor unless the concentration of the interfering element is at least 5 times the concentration of calcium. Interference may be compensated for by determining the concentration of the interfering element(s) in the unknown and by adding this concentration (± 20 percent) of the interfering element(s) to the standard solutions. In the determination of calcium in plant tissue, interference is seldom encountered.

Procedure

Prepare a series of standards containing 0, 1, 2, 3, and 4 meq./l. of calcium (0, 1, 2, 3, and 4 ml. of reagent D per 50 ml.) and the concentration of lithium chloride found to be optimum for the instrument.

Transfer an aliquot (usually 10 ml.) of the acid digest (Method 54a) containing 0.05 to 0.20 meq. of calcium to a 50-ml. volumetric flask and add the same concentration of lithium chloride used in the standard series. Add compensating solutions B, C, and E when necessary, and make to volume. Obtain instrument readings for standard solutions and unknowns with the wave-length indicator set at the point corresponding to the calcium emission maximum at 6,220 Å.

Calculations

Milliequivalents of Ca per 100 gm. of dry material = (meq./l. of Ca from calibration curve) \times 500/ml. in aliquot.

(55b) Calcium by Oxalate Method

The acid digest (Method 54a) may be analyzed for calcium by Method 77, if a flame photometer is not available.

Calculations

Milliequivalents of Ca per 100 gm. of dry material = normality of $KMnO_4 \times 10,000 \times$ (ml. of $KMnO_4$ - ml. of blank) / ml. in aliquot of acid digest.

(56) Magnesium

Remarks

Methods for determining magnesium by the use of thiazole yellow or related dyes have not given acceptable results at this laboratory. The following method, while time-consuming, has proved to be reliable. The calcium is removed as the oxalate, and magnesium is precipitated as magnesium ammonium phosphate hexahydrate. The phosphate is determined colorimetrically, and magnesium is calculated by reference to a calibration curve.

Apparatus

Photoelectric colorimeter or spectrophotometer.

Reagents

A. Oxalic acid, 1 N. Dissolve 63 gm. of oxalic acid in water and make to 1 liter.

B. Methyl orange, 0.01 percent in 95 percent ethanol.

C. Ammonium hydroxide, conc.

D. Hydrochloric acid (1+1).

E. Magnesium chloride, 5.00 meq./l. Dissolve 0.51 gm. $MgCl_2 \cdot 6H_2O$ in approximately 900 ml. of water. Standardize by Method 78 and add the calculated amount of water to bring the magnesium concentration to 5.00 meq./l.

F. Ammonium chloride, 3 percent. Filter before use.

G. Ammonium dihydrogen phosphate, 5 percent. Filter before use.

H. Phenolphthalein, 1 percent in 60 percent ethanol.

I. Ammonium hydroxide in ethanol and ether. Mix 20 ml. of concentrated ammonium hydroxide with 980 ml. of a mixture of equal volumes of ethanol, ether, and water.

J. Sulfuric acid (1+6).

K. Ammonium vanadate, 0.25 percent. Dissolve 2.5 gm. of ammonium vanadate in 500 ml. of boiling water, cool somewhat, and then add 60 ml. of reagent J. Cool to room temperature and dilute to 1 liter. Store in a brown bottle.

L. Ammonium molybdate, 5 percent. Store in a brown bottle.

Procedure

Transfer an aliquot (usually 10 ml.) of the acid digest from Method 54a containing 0.01 to 0.05 meq. of magnesium to a 25-ml. volumetric flask. Prepare a series of standards in 25-ml. volumetric flasks containing 0, 0.01, 0.02, 0.03, 0.04, and 0.05 meq. of magnesium (0, 2, 4, 6, 8, and 10 ml. of reagent E). To all standards and unknowns, add 3 drops of B,

acidify with D, if necessary, and add 1 ml. of D in excess. Add 1 ml. of A, heat to boiling, and neutralize with C. Cool and add more C if necessary to keep basic (yellow). Make to volume and filter through a dry filter paper; do not wash. Transfer a 5-ml. aliquot of the filtrate to a 15-ml. centrifuge tube and add 1 ml. each of F and G and 1 drop of H. Heat to about 90° C. in a water bath, and then add C until permanently pink. After 15 min., add an additional 2 ml. of C, stopper, mix, and let stand overnight.

Centrifuge at $RCF=2,000$ for 10 min., decant carefully, drain on filter paper for 10 min., and wipe the mouth of the tube with a clean towel or lintless filter paper. Suspend the precipitate and rinse the sides of the tube with a stream of 5 ml. of reagent I from a pipet equipped with a rubber bulb. Centrifuge at $RCF=2,000$, decant, drain for 5 min., and wipe the mouth of the tube. Repeat this washing procedure once.

Pipet 10 ml. of reagent J into the tube and twirl for a few seconds. After 5 min. transfer the contents quantitatively into a 100-ml. volumetric flask with a total of 50 ml. of water. Pipet 10 ml. each of K and L into the flask while swirling the solution rapidly. Make to volume and mix. After 10 min. measure the percent transmission of the unknown and standard solutions at 4,000 Å. or by means of an appropriate blue filter.

Calculations

Milliequivalents of Mg per 100 gm. of dry material = (meq. of Mg from calibration curve) $\times 10,000$ /ml. in aliquot of acid digest.

(57) Sodium

(57a) Sodium by Flame Photometer

Apparatus

Perkin-Elmer model 52 flame photometer with acetylene or propane burner.

Reagents

A. Lithium chloride, 0.05 N. Dissolve 2.12 gm. of lithium chloride in water and make to 1 liter.

B. Sodium chloride, 5.00 meq./l. Dissolve 0.2922 gm. of dry sodium chloride in water and make to 1 liter.

C. Potassium chloride, 12.5 meq./l. Dissolve 0.9320 gm. of dry potassium chloride in water and make to 1 liter.

D. Calcium chloride, 50.0 meq./l. Dissolve 2.503 gm. of pure calcium carbonate (calcite crystals) in 50 ml. of 3 N hydrochloric acid and dilute to 1 liter.

E. Magnesium chloride, 50.0 meq./l. Dissolve 5.1 gm. of $MgCl_2 \cdot 6H_2O$ in approximately 900 ml. of water. Standardize by Method 78 and add the calculated amount of water to bring the magnesium concentration to 50.0 meq./l.

Remarks

The optimum concentration of lithium chloride for use as an internal standard varies with individual flame photometers, but is usually 5 to 10 meq./l.

Sodium is frequently found in relatively small amounts in plant material, so that interference often occurs. Potassium interferes if the potassium : sodium ratio is 5 or greater; and calcium, if the calcium : sodium ratio is 10 or greater. Since these ratios are frequently exceeded in plant materials, it is advisable to determine calcium and potassium first, so that the approximate concentrations of interfering elements may be added to the sodium standard solutions. Magnesium does not cause interference unless magnesium : sodium ratios are in excess of 100. Such magnesium : sodium ratios are very rarely encountered.

Procedure

Prepare a series of standards containing 0, 0.1, 0.2, 0.3, and 0.4 meq./l. of sodium (0, 1, 2, 3, and 4 ml. of reagent B per 50 ml.) and the concentration of lithium chloride found to be optimum for the instrument.

Transfer an aliquot (usually 10 ml.) of the acid digest (Method 54a) containing 0.005 to 0.020 meq. of sodium to a 50-ml. volumetric flask and add the same concentration of lithium chloride used in the standard series. Add compensating solutions C, D, and E when necessary and make to volume. Obtain instrument readings for standard solutions and unknowns with the wave-length indicator set at the point corresponding to the sodium emission maximum at 5,890 Å.

Calculations

Milliequivalents of Na per 100 gm. of dry material = (meq./l. of Na from calibration curve) $\times 500$ /ml. in aliquot.

(57b) Sodium by Uranyl Zinc Acetate

Reagents

A. Uranyl zinc acetate. Weigh 300 gm. of uranium acetate dihydrate, 900 gm. of zinc acetate dihydrate, and 10 mg. of sodium chloride into a large flask. Add 82 ml. of glacial acetic acid and 2,618 ml. of water. Stir or shake until the salts are dissolved, leaving only a small amount of sodium uranyl zinc acetate precipitate. This may require several days. Filter before use.

B. Ethanol, saturated with sodium uranyl zinc acetate. Filter before use. Sodium uranyl zinc acetate may be prepared as follows: Add 125 ml. of reagent A to 5 ml. of 2 percent sodium chloride solution, stir, and after 15 min. collect the precipitate in a porous-bottomed porcelain crucible. Wash several times with glacial acetic acid, then several times with ether. Dry in a desiccator.

- C. Ether, anhydrous.
- D. Phenolphthalein, 1 percent in 60 percent ethanol.
- E. Calcium chloride dihydrate, 10 percent in water.
- F. Ammonium hydroxide (1+1).
- G. Acetic acid, 2 percent.
- H. Acetic acid, glacial.

Procedure

Transfer an aliquot (usually 25 ml.) of the acid digest (Method 54a) sufficient to give 50 to 200 mg. of sodium uranyl zinc acetate to a 50-ml. sugar flask. Add 1 drop of reagent D and 5 ml. of E and make the solution basic (pink) with F to precipitate phosphate. Make to 55 ml. and filter through a dry paper; do not wash. Transfer a 50-ml. aliquot to a 100-ml. beaker, acidify with H, and evaporate to dryness on a steam bath. Cool, dissolve the residue in 2 ml. of G, and add 75 ml. of filtered reagent A. Stir the solution and allow to stand for 1 hr. Filter through a porous-bottomed porcelain filtering crucible, taking care to transfer all the sodium uranyl zinc acetate precipitate onto the filter by means of a small wash bottle filled with A. Wash the beaker 5 times with 2-ml. portions of A and pass the washings through the filter. Allow the crucible to drain completely, because it is important to have the filter and the precipitate free of the reagent before washing with the alcohol. Wash the crucible 5 times with 2-ml. portions of B and, after removing all the alcohol by suction, wash once or twice with C. The suction is continued until the precipitate is dry. Allow the crucible to stand in a desiccator for 2 hr. and weigh.

Return the crucible to a suction apparatus and wash with small portions of water until all the soluble material is dissolved and passes through the crucible. Wash with alcohol and ether as above. Dry and weigh. The difference between the first and last weight represents the weight of sodium precipitate. The precipitate is assumed to have the composition: $(\text{UO}_2)_3\text{NaZn}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$; molecular weight, 1538.079; percent sodium, 1.4952.

Calculations

Milliequivalents of Na per 100 gm. of dry material = (gm. of sodium uranyl zinc acetate precipitate) \times 7,152/ml. in aliquot.

(58) Potassium

(58a) Potassium by Flame Photometer

Apparatus

Perkin-Elmer model 52 flame photometer with acetylene or propane burner.

Reagents

A. Lithium chloride, 0.05 N. Dissolve 2.12 gm. of lithium chloride in water and make to 1 liter.

B. Sodium chloride, 5.00 meq./l. Dissolve 0.2922 gm. of dry sodium chloride in water and make to 1 liter.

C. Potassium chloride, 12.5 meq./l. Dissolve 0.9320 gm. of dry potassium chloride in water and make to 1 liter.

D. Calcium chloride, 50.0 meq./l. Dissolve 2.503 gm. of pure calcium carbonate (calcite crystals) in 50 ml. of 3 N hydrochloric acid and dilute to 1 liter.

E. Magnesium chloride, 50.0 meq./l. Dissolve 5.1 gm. of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in approximately 900 ml. of water. Standardize by Method 78 and add the calculated amount of water to bring the magnesium concentration to 50.0 meq./l.

Remarks

The optimum concentration of lithium chloride for use as an internal standard varies with individual flame photometers but is usually 5 to 10 meq./l.

Interference by sodium occurs if the sodium : potassium ratio is 5 or greater, and by calcium if the calcium : potassium ratio is 10 or greater. Magnesium does not cause interference until the magnesium : potassium ratio is in excess of 100. Interference in the determination of potassium is very rarely encountered.

Procedure

Prepare a series of standards containing 0, 0.25, 0.50, 0.75, and 1.00 meq./l. of potassium (0, 1, 2, 3, and 4 ml. of reagent C per 50 ml.) and the concentration of lithium chloride found to be optimum for the instrument.

Transfer an aliquot (usually 10 ml.) of the acid digest (Method 54a) containing 0.01 to 0.05 meq. of potassium to a 50-ml. volumetric flask and add the same concentration of lithium chloride used in the standard series. Add compensating solutions B, D, and E when necessary and make to volume. Obtain instrument readings for standard solutions and unknowns with the wavelength indicator set at the point corresponding to the potassium emission maximum at 7,680 Å.

Calculations

Milliequivalents of K per 100 gm. of dry material = (meq./l. of K from calibration curve) \times 500/ml. in aliquot.

(58b) Potassium by Cobaltinitrite

Reagents

A. Nitric acid (1+15).

B. Trisodium cobaltinitrite, 20 percent. Store at about 5° C. and filter before use. The solution is stable for some time but should be prepared fresh at about biweekly intervals.

C. Nitric acid (1+1,500). Dilute 10 ml. of reagent A to 1 liter.

D. Ethanol, 95 percent.

E. Potassium chloride, 0.0100 N. Dissolve 0.7456 gm. of dry potassium chloride in water and make to 1 liter.

Procedure

Evaporate to dryness in a 50-ml. beaker an aliquot (usually 25 ml.) of the acid digest (Method 54a) containing 0.05 to 0.35 meq. of potassium. Add 10 ml. of water, 1 ml. of reagent A, and stir to dissolve. Add 5 ml. of B, stir and allow to stand for 2 hr. at 15° to 20° C. Filter in a porous-bottomed porcelain filtering crucible, the tare weight of which is known, using C in a wash bottle to make the transfer. Wash 10 times with C and 5 times with 2-ml. portions of D. Aspirate until quite dry. Wipe the outside with a cloth, dry for 1 hr. at 110° C., cool in a desiccator, and weigh.

Prepare a series of standards containing 0, 0.05, 0.15, 0.25, and 0.35 meq. of potassium (0, 5, 15, 25, and 35 ml. of reagent E) in 50-ml. beakers and proceed as directed for the aliquots of the acid digests.

Calculations

Milliequivalents of K per 100 gm. of dry material = (meq. of K from calibration curve) \times 10,000/ml. in aliquot.

(59) Chloride

Remarks

A modification of the method described by Clark and others (1942) has been found to give results in close agreement with those obtained by AOAC procedures with a considerable saving in time. The sample for chloride analysis together with a tube containing acid and a well containing base are placed in a tightly closed weighing bottle (fig. 32). The acid digests the filter-paper plug in the acid tube and reacts with the sample, volatilizing chloride as hydrogen chloride, which is absorbed by the potassium hydroxide. The absorbed chloride is then titrated with mercuric nitrate.

Apparatus

Make the following items from ordinary glass tubing:

1. Acid tube, approximately 1.0 cm. inside diameter, with one end drawn out to a capillary tip. Capacity, 2 ml.
2. Outer well, approximately 1.1 cm. inside diameter, 4 to 5 cm. long, sealed at one end.
3. Inner well, approximately 1.0 cm. outside diameter, 1 to 2 cm. long, sealed at one end.
4. Support for inner well of such length that when assembled, the top of inner well is at, or slightly above, the top of the outer well.

Clamp. Any screw-type clamp, equipped with rub-

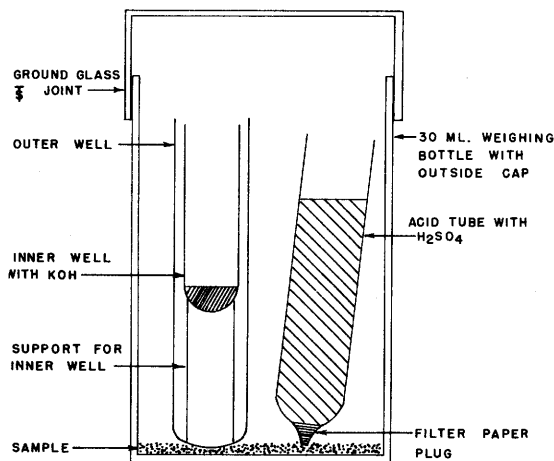


FIGURE 32.—Apparatus for the chloride determination (Method 59).

ber cushions which will hold the cap securely on the weighing bottle.

CAUTION.—After cleaning, rinse all glassware shown in figure 32 with nitric acid (1+100) and dry in oven. Use forceps, thereafter, in handling acid tubes, wells, and supports to avoid chloride contamination. Avoid contaminating the air with hydrogen chloride.

Reagents

- A. Potassium hydroxide, 50 percent.
- B. Sulfuric acid, conc.
- C. Ethanol, 95 percent.
- D. Diphenylcarbazone, 1 percent. Dissolve 1 gm. of diphenylcarbazone (Eastman No. 4459) in 100 ml. of 95 percent ethanol. Store at 5° C.
- E. Mercuric nitrate, 0.01 N. Dissolve 1.7 gm. of $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ in water to which 10 ml. of nitric acid (1+7) have been added and make to 1 liter. Store in brown bottle in the dark.
- F. Potassium chloride, 50.0 meq./l. Dissolve 3.728 gm. of dry potassium chloride in water and make to 1 liter.
- G. Hydrogen peroxide, 30 percent.
- H. Nitric acid (1+7).
- I. Bromphenol blue, 0.05 percent in ethanol.

Procedure

Weigh accurately in a 30-ml. weighing bottle a sample (usually 0.1 gm.) of dried plant material containing 0.02 to 0.25 meq. of chloride. To determine chloride in expressed sap, evaporate a suitable aliquot (usually 1 ml.) to dryness in the weighing bottle at 70° C. Prepare a series of standards containing 0, 0.05, 0.10, 0.15, 0.20, and 0.25 meq. of chloride (0, 1, 2, 3, 4, and 5 ml. of reagent F) and evaporate to dryness in the weighing bottles.

Plug the acid tube with macerated filter paper and assemble the apparatus as shown in figure 32. Add 3 drops of reagent A and 1 drop of C to the inner well. Add 2 ml. of B to the acid tube and moisten the ground surface of the cap with B. Seal the weighing bottle by twisting the cap into position and clamp securely. Place the assembly in an oven at 110° C. overnight. Remove, cool, and open the weighing bottles, using rubber gloves.

Transfer the inner well with forceps to a porcelain casserole and add 5 ml. of water. Add 3 drops of reagent I, neutralize with H, dissolving all of the residue in the inner well, and add 1 drop of H in excess. Add 3 drops of G, 1 drop of D, and titrate with E to a purple or pink color, depending on volume. Prepare a standard curve; it is not linear over the entire range.

Calculations

Milliequivalents of Cl per 100 gm. dry material = (meq. of Cl in sample) \times 100/gm. of sample.

Milliequivalents of Cl per liter of sap = (meq. of Cl in aliquot) \times 1,000/ml. in aliquot.

References

Association of Official Agricultural Chemists (1950, 6.41-6.46, p. 105, 106), Clark and others (1942).

(60) Sulfur

Reagents

- A. Hydrochloric acid, conc.
- B. Barium chloride, 10 percent. Filter before use.
- C. Methyl orange, 0.05 percent in ethanol.

Procedure

Transfer an aliquot (usually 50 ml.) of the digest (Method 54b) containing 0.1 to 2.0 meq. of sulfate to a 250-ml. beaker. Add 2 drops of reagent C, acidify with A if necessary, and then add 1 ml. of A. Make to approximately 100 ml. with water, heat to boiling, and add an excess of B, drop by drop, with constant stirring. Cover with a watchglass and allow to stand on a steam bath for several hours, until the volume is reduced to approximately 50 ml. After cooling, filter the precipitate of barium sulfate through an ignited and weighed Gooch crucible and wash with water until free of chloride. Dry, ignite in a muffle at low red heat, cool, and weigh. Ashless filter paper may be used in place of the Gooch crucible, but the muffle in which the ignition is made must be well ventilated.

Calculations

Milliequivalents of total S (expressed as sulfate) per 100 gm. of dry material = gm. $\text{BaSO}_4 \times 42,841/\text{ml. in aliquot}$.

(61) Phosphorus

Phosphorus is retained quantitatively by both the magnesium nitrate ignition and wet digestion, provided the perchloric acid digest is not allowed to go to dryness.

Apparatus

A photoelectric colorimeter or spectrophotometer.

Reagents

- A. Sulfuric acid (1+6).
- B. Ammonium vanadate, 0.25 percent. Dissolve 2.5 gm. of ammonium vanadate in 500 ml. of boiling water, cool somewhat, and add 60 ml. of reagent A. Cool to room temperature and dilute to 1 liter. Store in a brown bottle.
- C. Ammonium molybdate, 5 percent.
- D. Potassium dihydrogen phosphate, 2.50 millimoles/l. Dissolve 0.3404 gm. of potassium dihydrogen phosphate in water and make to 1 liter.

Procedure

Transfer an aliquot (usually 5 ml.) of the solution prepared by Method 54a or 54b containing 0.002 to 0.020 millimoles of phosphate to a 50-ml. volumetric flask. Prepare a series of standards containing 0, 0.005, 0.010, 0.015, and 0.020 millimoles of phosphate (0, 2, 4, 6, and 8 ml. of reagent D). Add to each flask containing unknown or standard, 5 ml. each of A, B, and C successively, shaking the flask during each addition. Make to volume, allow to stand 15 to 30 min., and determine transmittance at 4,000 Å., or by means of a suitable blue filter.

Calculations

Millimoles of phosphate per 100 gm. of dry material = (millimoles of phosphate from calibration curve) $\times 10,000/(\text{ml. in aliquot} \times \text{gm. of sample digested})$.

1 millimole of phosphate = 1 meq. of H_2PO_4^- .

(62) Boron

Remarks

Leaf samples, collected and prepared according to Method 53, are usually the best index to the boron status of the plant.

Apparatus

A spectrophotometer or photoelectric colorimeter.

Alkali-resistant (boron-free) glassware, porcelainware, platinum, or fused-quartz dishes. Avoid the use of borosilicate glassware.

Reagents

- A. Calcium oxide, powdered.
- B. Hydrochloric acid (1+1).
- C. Hydrochloric acid, conc.
- D. Sulfuric acid, conc.
- E. Carmine, 0.05 percent by weight in conc. sulfuric acid (0.920 gm./l.). Shake until completely dissolved.
- F. Boric acid, 100 p. p. m. boron. Dissolve 0.5716 gm. of boric acid in water and make to 1 liter.

Procedure

Weigh a portion of the dry sample (usually 2.000 gm.) containing not more than 1.00 mg. of boron and transfer to a porcelain casserole or platinum dish. Add 0.1 gm. of reagent A per gram of sample and mix well. Ignite as completely as possible in a muffle at 500° to 550° C., cool, and moisten with water. Cover with a watchglass and introduce 3 ml. of B per gram of sample, which should make the solution strongly acid. Heat on a steam bath for 20 min. Transfer quanti-

tatively to a 100-ml. volumetric flask, make to volume with water, and filter through a dry filter paper.

Prepare a series of standard solutions containing 0 to 10 p. p. m. boron (0 to 1.00 mg./100 ml.) by diluting 0, 2, 4, 6, 8, and 10 ml. of reagent F to 100 ml. Pipet 2 ml. of each of the standards and of the unknowns into Erlenmeyer flasks. Add 2 drops of C to each standard and 2 drops of water to each unknown. Add 10 ml. of D to each Erlenmeyer flask, mix, and cool. Add 10 ml. of E, mix, and allow to stand at least 45 min. for color development. Determine the transmittance at 5,850 Å., or by means of a suitable yellow filter.

Calculations

Parts per million B in dry plant material = (p. p. m. B from calibration curve) \times 100/gm. of sample.

Reference

Hatcher and Wilcox (1950).